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Immunosuppression-induced susceptibility of inbred hamsters (Mesocricetus auratus) to lethal-disease by lymphocytic choriomeningitis virus infection

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Summary

The role of the immune response in the pathogenesis of lethal and non-lethal lymphocytic choriomeningitis virus (LCMV)-infections of young adult Syrian golden hamsters (*Mesocricetus auratus*) of different strains was examined using immunosuppressive treatment with cyclophosphamide or with whole-body gamma-irradiation. In all hamsters, the LCMV strains, WE and Armstrong (ARM), caused systemic infections and induced comparable serum LCMV-antibody titers. However, lethal wasting-disease occurred which was hamster-strain and virus-strain dependent. With WE-inocula, MHA and PD4 inbred hamsters were all susceptible to lethal-disease and failed to completely eliminate infection. All LSH and CB inbred hamsters resisted lethal-disease and totally cleared WE-infection. Random colony-bred LVG hamsters and inbred LHC hamsters were intermediate in WE-susceptibility; some died with wasting, while others survived with minimal to no illness. ARM was avirulent for all hamsters and infections were totally cleared. By immunosuppressive treatment, all hamsters were rendered completely susceptible to lethal-disease by WE, and had unresolved infections and diminished serum LCMV-antibody titers. Immunosuppression also rendered all hamster strains partially susceptible to lethal infection by ARM. The hamster immune response was thus shown to suppress LCMV-infection and protect against lethal illness.

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Introduction

The Syrian golden hamster (*Mesocricetus auratus*) has been a long recognized host for lymphocytic choriomeningitis virus (LCMV) and of considerable concern as a reservoir for human LCMV-outbreaks (1, 7, 8, 15, 29, 33, 41, 42, 44, 47, 50]. However, past information on LCMV-virulence for hamsters has not been consistent. While many studies have reported inapparent, self-limited and immunizing infections [8, 15, 29, 33, 41, 42, 44, 47, 50], a few have given indications that lethal and non-lethal illness can occur [8, 29, 44, 47].

Pathogenesis and host-virus parameters are not clearly known for hamster LCMV-infections. Perhaps conflicting data regarding LCMV-lethality for hamsters discouraged the use of these rodents for more detailed investigations. Nevertheless, several discussions and reviews have implied that immunopathogenetic mechanisms underlie the clinical outcome of these infections [1, 7, 8, 15, 23, 29]. By this process, infectious-virus elimination by an anti-viral immune response would occur with disease and death; the absence of this immune response should favor infectious-virus persistence without acutely fatal consequences. A contrasting view has argued that anti-viral immunity mediates virus clearance and thereby protects hamsters from disease and mortality by LCMV [29, 42, 47].

In the past there has been no reliable experimental host-virus system to adequately assess the immune response role in the pathogenesis of hamster LCMV-infections. However, recently, uniform LCVM-hamster models of inapparent, non-lethal infections and lethal infections with wasting-disease were established in our laboratory [10]. The LCMV strains, WE and Armstrong (ARM), produced systemic infections in all young adult hamsters. Also, hamsters of all tested background strains responded to infection with comparable serum LCMV-antibody titers. Lethality and disease were dependent upon both hamster-strain and virus-strain. The LCMV strain, WE, was hamster-virulent. Inbred MHA and PD4 hamsters were susceptible to lethal wasting-disease by WE. Inbred LSH and CB hamsters were resistant to illness and death by WE. Random colony-bred LVG hamsters and inbred LHC hamsters were intermediate, or partial, in susceptibility to lethal WE-infection; some died of wasting-disease, while others recovered from infection with minimal to no overt disease signs. The LCMV strain, ARM, was avirulent for all young adult hamsters, causing only on inapparent and resolving infection. All hamsters that survived infection by ARM or WE appeared healthy, had high serum LCMV-antibody titers, and were aviremic with tissues free of infectious-virus. These LCMV-survivors also resisted secondary WE-challenge. Due to the uniform hamster strain-dependent mortality

responses to LCMV, these host-virus systems of lethal and non-lethal infections ought to be reliable for further experimental pathogenesis and immunity studies [30].

From descriptive accounts of lethal and non-lethal LCMV-infections in hamster of the different strains, it was not clear whether disease and death were a consequence of immunopathogenetic mechanisms, and to what extent the immune response was critical for hamster survival from LCMV-infections. To better understand the immune response role in the clinical outcome of hamster LCMV-infections, experiments were conducted to determine the effects of immunosuppression on the susceptibility of inbred hamsters to mortality, disease, and infection by the virulent, WE, and avirulent, ARM, strains. For immunosuppressive treatment of animals two modalities, cyclophosphamide [43] and whole-body gamma-irradiation [2], were applied.

Material and methods

Virus stocks

LCMV strains WE [35] and ARM [3] of recorded passage histories (22, 34) were obtained from Dr. W. E. Kirk (Dept. of Microbiology, Medical Center, West Virginia University, Morgantown, WV). Both LCMV strains were plaque purified, propagated, and stored at −70 °C as previously described [10]. Parental stocks of LCMV were similarly prepared without plaque purification. Plaque purified WE and ARM were used for all animal inoculations. The virulence and in vitro growth traits of these virus preparations were previously described [10].

Plaque assay for infectious-LCMV

As previously described [10], the LCMV-infectivity of all virus samples, and bloods and organ homogenates from LCMV-inoculated animals was measured in plaque-forming units (PFU) in a Vero-cell monolayer-agarose overlay culture [7, 16]. To develop LCMV plaques for counting, a second medium-agarose overlay with neutral red was applied to the cultures [10, 34]. LCMV-infectivity was expressed as mean $\log_{10}$ PFU ± 1 standard error of the mean (SEM) per gram or ml of sample. Lowest detectable infectious-titers for samples of blood and tissue homogenates were, respectively, 0.7 $\log_{10}$ PFU/ml and 1.7 $\log_{10}$ PFU/gram.

Animals and preparation of tissue samples for virologic and immunologic assays

Female Syrian golden hamsters (*Mesocricetus auratus*) reared at the Lakeview Hamster Colony (Newfield, NJ), were purchased at 8–10 weeks of age from Charles River Breeding Laboratories (Wilmington, MA). Strains used were CB/SsLak, LHC/Lak, LSH/SsLak, MHA/SsLak, PD4/Lak, and the random colony-bred Lak:LVG (SYR). The strains are respectively referred to as CB, LHC, LSH, MHA, PD4, and LVG. Although the genetic homogeneity of these animals was not monitored, the pedigree of these hamster strains is assured by the commercial supplier.

Animals were housted in P-3 laboratory containment facilities in filter-topped cages, fed and watered ad libitum. Prior to use all animals were tested and found to be sero-negative for LCMV.

Hamsters were inoculated in the peritoneum with 3.3 $\log_{10}$ PFU of either WE or

ARM in 0.2 ml, and were routinely monitored for body weight, signs of illness, and mortality. Blood samples were drawn from the retro-orbital sinus into heparinized tubes for infectious-virus assay and stored at —70 °C. Serum for LCMV-antibody assays was collected and stored at —20 °C. For infectious-virus assay organs of sacrificed animals were aseptically weighed, homogenized, clarified and stored at —70 °C.

LCMV-Antibody assays

Sera were heated at 56 °C for 30 min and tested, as previously described [10], for LCMV-antibody titers by an indirect immunoflourescent antibody test (IFAT) and an in vitro assay for neutralizing-antibody used for arenaviruses [16, 32]. Briefly, in the IFAT assay, dilutions of test sera were incubated with slides of acetone-fixed, LCMV-infected, Vero-cells and with slides of fixed uninfected control, Vero-cells. Slides were washed, reacted with a prediluted FITC-IgG conjugate of goat anti-hamster gamma globulin (Cappel Laboratories, Cooper-Biomedical Inc., Malverne, PA), washed again, mounted with PBS-glycerol and a coverslip, and examined for immunofluorescence. End-point serum IFAT titer is the highest dilution showing definite cytoplasmic granular fluorescence of infected cells and is expressed as the mean reciprocal $\log_{10}$ dilution ± 1 standard error of the mean (SEM) for sample groups. Samples that were negative for antibody at an initial 1 : 5 dilution were recorded with IFAT ($-\log_{10}$) titers of 0.7.

LCMV-neutralizing antibody was measured in a plaque reduction test by a constant-serum varied-virus protocol [16]. It was performed using the Vero plaque assay to titrate a standard LCMV preparation in the presence of a constant 1 : 5 test serum dilution and 10% fresh normal guinea pig serum as complement source [10]. This titration was always conducted in parallel with a virus control in which the infectious titer of the standard LCMV was determined in the absence of any test serum. The standard LCMV preparation used for this procedure was a parental WE stock with a 7.3 Log_{10} PFU per ml titer. Final infectivity titers of the standard WE virus in each test serum and virus control were used to calculate serum LCMV-neutralizing antibody titers as follows: [($\log_{10}$ PFU of virus control) minus ($\log_{10}$ PFU in test serum)]. LCMV-neutralizing antibody titer was expressed as a mean $\log_{10}$ neutralization index (LNI) ± 1 SEM for experimental animal groups. Serum samples that were nagative for LCMV-neutralizing antibody were recorded with LNI titers of 0.7.

Immunosuppression protocols

Immunosuppression of hamsters was achieved either by whole-body gamma-irradiation or by cyclophosphamide administration. One day before LCMV-inoculation, hamsters received 600 rad whole-body irradiation, from a ^{137}Cs-source gamma-irradiator (Gammacell 40; Atomic Energy of Canada Ltd., Ottawa, Ontario, Canada). A total of 340 mg cyclophosphamide (Cytoxan; Mead Johnson and Co., Evansville, IN) per kg of hamster body weight was given in multiple intraperitoneal injections as such: 200 mg per kg one day before LCMV, 100 mg per kg 3 days after virus, and 40 mg per kg 8 days after virus.

Results

The initial objective of this study was to establish an immunosuppressive regimen that would protect or prolong the survival of hamster strains that are completely susceptible (MHA and PD4) or partially susceptible (LVG and LHC) to fatal illness by WE-infection. Using 450 rads whole-body gamma-irradiation and varied cyclophosphamide regimens

(i.e. single or multiple injections of doses totaling up to 300 mg drug per kg body weight, given 1 day before virus to 3 days after virus) this goal was not accomplished. Instead, the survival and serum LCMV-antibody titers of hamsters of intermediate disease-susceptibility (LVG and LHC) and disease-resistant inbreds (LSH and CB) were reduced. More of these animals had lethal wasting-disease marked by dehydration, lethargy, body weight loss land diarrhea [10]. In addition, blood LCMV-infectivity levels of treated animals were higher than those of untreated animals (data not shown).

These studies were continued using more intensive immunosuppressive regimens. The mortality of normal animals that received 600 rad whole-body irradiation or cyclosphosphamide (see Materials and methods) was less than 10% over a 60 day observation period. Immunosuppression of susceptible MHA and PD4 hamsters did not prevent lethal-disease after WE-inoculation (data not shown). The duration of survival was similar among untreated (17.3 ± 5.0 days), irradiated (14.9 ± 5.3 days), and cyclosphosphamide treated (14.5 ± 5.3 days) MHA hamsters after WE-infection. Clinical disease with a progressive 30% to 55% body weight loss was noted in all these untreated and immunosuppressed hamsters after WE-inoculation (data not shown). Furthermore, serum LCMV-antibody titers of the WE-inoculated MHA and PD4 hamsters were affected by the immunosuppressive treatments. In untreated animals IFAT titers of 1.5 to 2.0 ($-\log_{10}$) were usually detected in the sera by 7 days after WE-inoculation and these titers increased to 3.0 ($-\log_{10}$) by day 14. None of the hamsters, treated with the immunosuppressive agents, had detectable serum titers of IFAT when sampled on or after day 7 of WE-inoculation (data not shown). Blood samples taken form these animals, between 7 and 14 days, all had comparable levels of infectious-virus ranging form 5.0 to 6.0 $\log_{10}$ PFU per ml (data not shown).

The effect of treatment with either irradiation or cyclophosphamide on mortality, immunity and infection by WE in LCMV-disease resistant inbred LSH (Tables 1 and 2, and Fig. 1) and CB (data not shown) hamsters was examined. As shown in Table 1, without treatment such animals after WE-inoculation did not die and never had signs of illness. By 42 days after WE-inoculation these animals underwent a 29% body weight gain which was comparable to the weight increment of the healthy age-matched uninoculated controls (Table 1). Humoral LCMV-immunity was also induced in these surviving hamsters: mean serum LCMV-antibody titers, IFAT and LNI, were respectively 3.8 ($-\log_{10}$) and 2.6. These survivors were also not viremic.

Treatment with either irradiation or with cyclophosphamide rendered all LSH (Table 1) and CB (data not shown) hamsters susceptible to lethal wasting-disease by WE. Mean times to death after WE-inoculation for

Table 1. Effect of immunosuppression on the survial of LSH hamsters after intraperitoneal WE-inoculation[a]

Inoculated virus[b]	Survival ratio[c]	Survival time[d]	Body weights[e]			LCMV-antibody[f]	
			initial	dead	survivors	IFAT	LNI
Immunosuppressive treatment: None							
+	9/9	**	91.6±3.3	–	118.3±6.9	3.8±.3	2.6±.4
–	7/7	**	89.1±4.1	–	114.6±8.0	⩽ 0.7	⩽ 0.7
Immunosuppressive treatment: Irradiation							
+	0/14	17.7±4.4	90.4±4.8	50.3±9.6	–	–	–
–	8/8	**	88.6±4.3	–	112.7±7.7	⩽0.7	⩽0.7
Immunosuppressive treatment: Cyclophosphamide							
+	0/16	18.6±3.7	92.6±3.7	54.9±9.1	–	–	–
–	12/12	**	91.6±3.5	–	113.0±4.8	⩽ 0.7	⩽ 0.7

[a] Inbred LSH hamsters are resistant to lethal wasting-disease by WE. (Similar results were obtained for diseases-resistant inbred CB hamsters)

[b] Inoculated virus: Groups of animals given 3.3 $\log_{10}$ PFU of WE by the intraperitonal route are shown by the postitive sign (+). Groups of animals as uninoculated controls are shown by the negative sign (–)

[c] Number of animals alive at 42 days to the total number inoculated

[d] Mean time to death for animals with fatal WE-inoculation is expressed as days (±1 SD).

[e] *initial* Mean body weight of animals in experimental groups at time of virus inoculation. *dead* Mean body weight of lethally inoculated animals at death. *survivors* Mean body weight of animals alive at 42 days. Weights are expressed as grams (±1 SD)

[f] Mean IFAT ($-\log_{10}$) and LNI serum LCMV-antibody titers (±1 SEM) of surviving animals at 42 days. Bloods of all animals surviving beyond 42 days of WE-inoculation were negative for infectious-LCMV. Serum titers of ⩽ 0.7 are negative for LCMV-antibody

** Surviving hamsters lived beyond 42 days of virus inoculation

irradiated hamsters and for cyclophosphamide-treated hamsters were 17.7 days and 18.6 days, respectively. All these lethally inoculated hamsters had wasting-disease signs that resembled the illness of disease-susceptible hamsters. WE-inoculated LSH hamsters, treated with irradiation, lost 41.4% body weight by the time of death. A 40.7% reduction in mean body weight was noted by time of death for WE-inoculated, cyclophosphamide treated, LSH hamsters.

The effect of immunosupressive agents on the virologic and immunologic status of WE-inoculated, disease-resistant, LSH (Table 2 and Fig. 1) and CB (data not shown) hamsters was examined. Animals were sacrificed 21 days after WE-inoculation. Without immunosuppressive treatment WE-inoculated LSH (Table 2) and CB (data not shown) hamsters had no disease signs and gained as much body weight as did uninoculated animals. These animals were barely viremic; infectious-virus blood levels

Table 2. Effect of immunosuppression on WE-infection of LSH hamsters[a]

LCMV inoculation[b]	No. of animals[c]	Body weights[d] day 0	Body weights[d] day 21	Viremia[e]	LCMV-antibody[f] IFAT	LCMV-antibody[f] LNI
Immunosuppressive treatment: None						
+	8	91.8±4.6	108.2±7.6	1.2±0.7	3.2±0.3	1.2±0.2
−	8	89.6±4.2	103.5±6.7	⩽ 0.7	⩽ 0.7	⩽ 0.7
Immunosuppressive treatment: Irradiation						
+	12	91.1±4.8	58.0±11.5	5.6±0.8	0.9±0.4	⩽ 0.7
−	6	90.8±3.3	106.4±7.4	⩽ 0.7	⩽ 0.7	⩽ 0.7
Immunosuppressive treatment: Cyclophosphamide						
+	12	91.6±4.3	55.3±8.2	5.9±0.6	0.9±0.4	⩽ 0.7
−	8	91.7±5.4	104.8±7.7	⩽ 0.7	⩽ 0.7	⩽ 0.7

[a] Inbred LSH hamsters are resistant to lethal infection by WE. (Similar results were obtained for lethal disease-resistant inbred CB hamsters)

[b] Groups of animals given 3.3 $\log_{10}$ PFU of WE by the intraperitonal route are shown by the postitive sign (+). Groups of animals as uninoculated controls are shown by the negative sign (−)

[c] Numbers of individuals in each experimental group sacrificed on day 21 of WE-inoculation

[d] Mean body weight is expressed in grams (± 1 SD)

[e] Mean infectious-virus content of blood for each experimental group at 21 days is expressed as $\log_{10}$ PFU (± 1 SEM) per ml blood. Virus titers of ⩽ 0.7 $\log_{10}$ PFU are negative for infectivity

[f] Mean IFAT ($-\log_{10}$) and LNI serum LCMV-antibody titer (± 1 SEM) of each experimental group at day 21. Serum titers of ⩽ 0.7 are negative for LCMV-antibody

of LSH (Table 2) and CB (data not shown) hamsters were respectively 1.2 and ⩽ 0.7 $\log_{10}$ PFU per ml. Seroconversion to LCMV was also evident with IFAT titers > 3.0 ($-\log_{10}$) and low LNI titers of about 1.1 to 1.2. However, by treatment with irradiation or cyclophosphamide, the LCMV-immune response of WE-inoculated hamsters was depressed as shown by a lack of IFAT and LNI serum titers. All these later animals were ill with significant body weight reduction. Moreover, all these immunosuppressed hamsters were highly viremic with about 5.5 $\log_{10}$ PFU of infectious-LCMV per ml of blood.

The effect of immunosuppressive treatment on the extent of systemic infection was also determined at day 21 for disease-resistant inbred LSH (Fig. 1) and CB (data not shown) hamsters. By 1 week after WE-inoculation infectious-LCMV can be readily detected in the bloods and organs of all hamsters, and by 3 weeks infectivity is reduced to barely detectable titers in disease-resistant strains [30; Genovesi and Peters, submitted for publication]. By 21 days, as shown in Fig. 1 for untreated, WE-infected

LSH hamsters, organ-virus titers were either undetected ($\leqslant 1.7$ $\log_{10}$ PFU per gram) or no higher than 2.0 $\log_{10}$ PFU per gram. Infectious-virus levels in all tested organ homogenates of irradiated hamsters and cyclophosphamide-treated hamsters were significantly higher than for

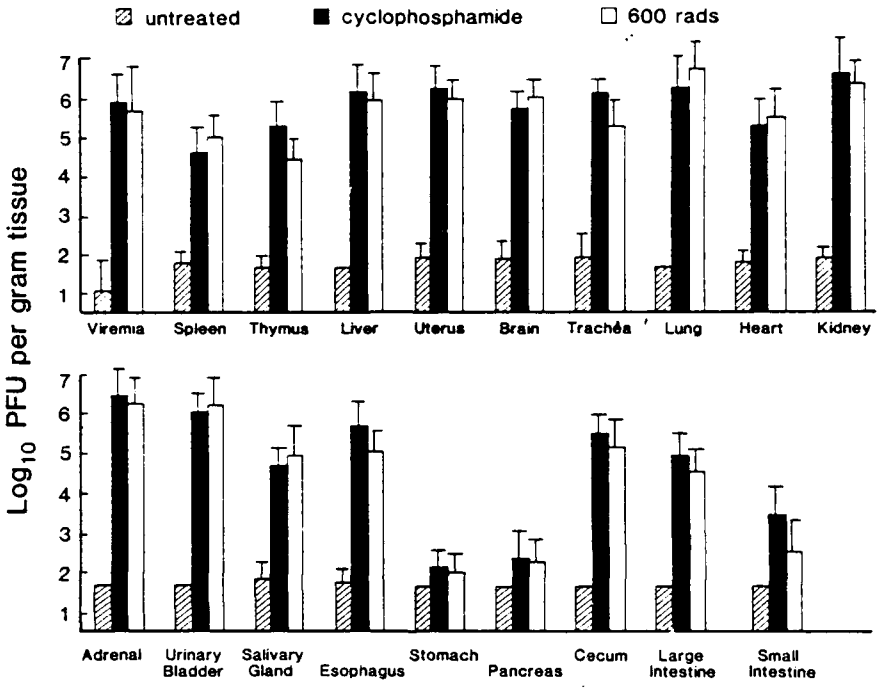

Fig. 1. Effect of immunosuppression on the infectious-LCMV contents of organs of WE-infected, disease-resistant LSH hamsters. Treated and untreated female LSH hamsters were inoculated in the peritoneum with 3.3 $\log_{10}$ PFU of WE and sacrificed 21 days later. Bloods and organs were tested for infectious-LCMV. Observations were based on 8 animals per treatment group. Infectious-virus titers are the mean $\log_{10}$ PFU (± 1 SEM) per ml blood or per gram of organ. Virus titers of $\leqslant 1.7$ $\log_{10}$ PFU per gram for organs and $\leqslant 0.7$ $\log_{10}$ PFU per ml for blood were considered negative for infectivity. (Comparable results were also obtained for WE-disease resistant CB hamsters)

the untreated WE-infected animals, and contained 5.0 to 6.0 $\log_{10}$ PFU per gram.

For hamsters of strains (LVG and LHC) intermediate in susceptibility to lethal wasting-disease by WE, 60% to 70% died with wasting-disease between 1 to 4 weeks after 3.3 $\log_{10}$ PFU challenge inoculum. The remaining animals did not have overt signs of illness and survived beyond 4 weeks of virus inoculation with no infectious-virus in their tissues [10]. As reported for the other strains, the immunosuppressive treatments

rendered all LVG and LHC strain hamsters completely susceptible to lethal wasting-disease (data not shown). Following WE-inoculation all these hamsters died and lost 35% to 50% of original body weight. Time to death in these untreated hamsters with lethal WE-inoculation ranged

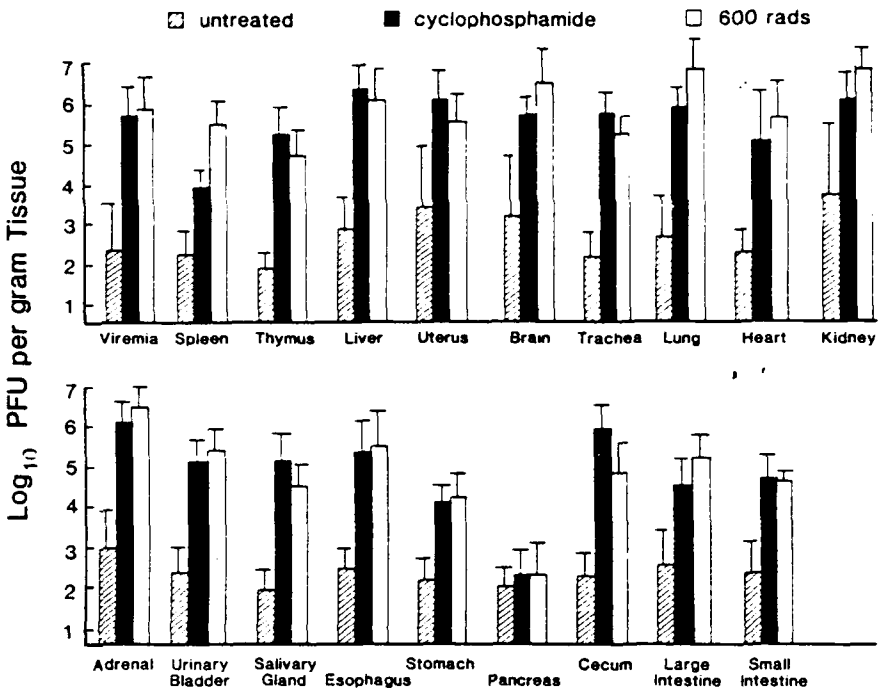

Fig. 2. Effect of immunosuppression on the infectious-LCMV contents of organs of WE-infected LVG hamsters of intermediate susceptibility to lethal wasting-disease by WE. Organ-virus titers of treated and untreated female LVG hamsters are shown at 21 days after WE-inoculation. See legend of Fig. 1 for other details. (Comparable results were also obtained for inbred LHC hamsters of intermediate susceptibility to lethal WE-disease)

from 12 to 25 days. All immunosuppressed hamsters died with comparable survival times ranging from 9 to 22 days after WE-inoculation.

Effects of immunosuppressive treatment on LCMV-infection and immunity in LVG and LHC strain hamsters were similar to those for the aforementioned inbred hamsters. WE-inoculated LVG and LHC hamsters, that were treated with the immunosuppressive agents, failed to develop serum LCMV-antibody titers (data not shown). Moreover, compared to untreated, WE-inoculated LVG and LHC hamsters, infectious-virus titers persisted at higher levels in the tissues of the immunosuppressed counterparts (data not shown). This is also illustrated in Fig. 2

Table 3. Effect of immunosuppression on hamster survival after intraperitoneal ARM-inoculation

Hamster[a] strain	Immunosuppressive treatment	Lethal[b] ratio	Survival[c] time
MHA	None	0/9	
	Irradiation	12/16	15.2 ± 4.9
	Cyclophosphamide	13/16	13.9 ± 4.3
LVG	None	0/7	
	Irradiation	9/16	15.1 ± 3.8
	Cyclophosphamide	11/16	16.4 ± 3.7
LSH	None	0/8	
	Irradiation	6/16	14.7 ± 4.5
	Cyclophosphamide	12/16	15.6 ± 5.9

[a] Animals were given 3.3 $\log_{10}$ PFU of ARM. Immunosuppressive treatments were as indicated

[b] Number of animals dead by 42 days after virus inoculation to the total number inoculated

[c] Mean time to death for animals with fatal virus inoculation is expressed as days (± 1 SD) and does not include survivors. All hamster survivors lived beyond 42 days of ARM-inoculation

for LVG hamsters at 21 days of WE-infection. Organs and bloods of WE-infected LVG hamsters, that were treated with either irradiation or with cyclophosphamide, contained significantly more infectious-LCMV ($\geq$ 2.0 to 3.0 $\log_{10}$ PFU per gram or per ml) than did untreated, WE-infected hamsters.

The effect of immunosuppressive treatment on the susceptibility of inbred hamsters to mortality by the avirulent ARM strain of LCMV was also tested (Table 3). Without treatment all MHA, LVG, and LSH hamsters (Table 3), as well as PD4, LHC, and CB strain hamsters (data not shown) survived ARM-inoculation. However, immunosuppressive treatment rendered all these hamster strains partially susceptible to lethality by ARM. No more than 80% of the immunosuppressed hamsters died following ARM-inoculation, and mean survival times ranged from 2.0 to 2.5 weeks.

It should also be noted uninfected hamsters treated with irradiation or cyclophosphamide appeared healthy throughout the periods of observation, and only an occasional animal died. Body weight measurements on day 21 and on day 42 confirmed that immunosuppressed hamsters of all six inbred strains had gained as much weight as did age-matched, untreated controls (refer to control data in Tables 1 and 2).

Discussion

In nature arenaviruses usually persist in chronically infected rodent hosts. Virus transmission from rodent reservoirs to susceptible hosts of similar or other mammalian species could lead to infections with a spectrum of consequences ranging from no apparent disease to acutely fatal or chronic disease. Much of our understanding of arenavirus virulence has relied on the murine-LCMV paradigms of acute and chronic immunopathology [7, 11, 23, 25]. In acutely lethal murine LCMV-diseases, T-lymphocyte immune-effector mechanisms are involved in virus elimination from infected tissues, and also lead to illness and death [7, 23–25, 45, 46]. Under conditions of compromised thymus-dependent immune functions LCMV-infected murine hosts survive and remain actively infected as life-long virus carriers. This latter condition may develop after congenital or neonatal infection [7, 23, 26], by infection of genetically athymic mice [7, 27], or in immunocompetent adults by immunosuppressive treatment [7, 13, 14, 38–40]. In LCMV-carrier mice chronic immune-complex disease occurs [7], and the extent to which pathology and disease develop may be due to thymus-dependent LCMV-antibody responses [7, 37].

Whereas immunosuppressive treatment protected susceptible murine hosts from immunopathogenetic disease by LCMV-infection [6, 13, 14, 38–40], such measures had an opposite effect on the susceptibility of hamsters to WE-induced mortality. By treatment with either irradiation or cyclophosphamide all hamsters, regardless of pedigree, died of WE-infection and exhibited diarrheal wasting-disease that characterized lethal WE-infections of susceptible inbred MHA and PD4 hamsters [10]. The regimes of irradiation and cyclophosphamide used in this study suppressed serum LCMV-antibody titers in WE-inoculated MHA and PD4 hamsters, without affecting mortality, infection, and clinical illness patterns (data not shown). These results demonstrated that immune-mediated pathogenesis was not critical in the fatal wasting-disease of susceptible hamsters with WE-infection. The host immune response clearly functioned to protect infected, disease-resistant hamsters against lethal illness.

Infection by the avirulent ARM strain of LCMV did not cause mortality (Table 3) or overt illness in any young adult hamster. These animals eliminated ARM-infection and developed LCMV-immunity [10]. In immunosuppressed hamsters ARM-inoculation induced death in no more than 80% of the animals (Table 3). These results suggest that although the immune response was an important contributing factor to the nonlethal outcome of ARM-infection, it was not the sole determinant. Observations on virus replication in irradiated and cyclophosphamide treated hamsters (Figs. 1 and 2) are also pertinent to understanding the

mechanisms that underlie the resistance of some inbred hamster strains to WE [10]. Viremia appears within a few days of WE-inoculation and reaches a peak by 1 week, at which time IFAT-antibody is present in serum. Viremia and organ-associated infectious-virus were higher and more prolonged in WE-disease susceptible hamster strains, but the pattern of virus distribution in bloods and organs was basically the same in all animals [30; Genovesi and Peters, submitted for publication]. When WE-disease resistent animals, treated with irradiation or cyclophosphamide, were monitored after virus-inoculation, their LCMV-antibody responses were markedly depressed and viremia levels were significantly elevated (Table 2, Figs. 1 and 2). The reported virus-infectivity titers in the bloods and organs of the WE-infected immunosuppressed MHA hamsters approximated levels found in WE-infected, disease-susceptible MHA hamsters [30; Genovesi and Peters, submitted for publication]. Thus, even the tissues of WE-disease resistant hamster strains allow for virus replication to high levels, if the host immune response is ablated. The mechanism(s) of hamster resistance to LCMV lies in the immune system, although not necessarily in the humoral antibody response as measured in this and other studies [10, 30]. Although before death, WE-disease susceptible hamsters produce LCMV-antibodies at titers that are comparable to those of disease-resistant hamsters, such antibodies may not be protective. High infectious-LCMV titers in bloods and organs always correlated with induction of wasting and death, whether comparing lethal WE to avirulent ARM, susceptible to resistant hamster strains, or immunosuppressed to immunointact animals. This relationship was noted in previously reported LCMV-infections of hamsters [29], in other arenavirus disease models [3, 6, 17–19, 30, 31, 36, 48, 49], and in human Lassa fever virus infections [20].

The role of antibody in recovery from infections with viruses such as LCMV, Lassa, and Pichinde is not clear. Certainly the appearance of antibodies measured by IFAT in the LCMV-infected hamster or in Lassa fever virus infections [17, 19, 20] does not herald any significant change in disease progression. Although it can be argued that the lethal effects of hamster LCMV-infections are due to the levels of infectious-virus in the tissues, the simultaneous presence of viremia and circulating virus-specific antibody suggests that there may be a secondary role for immunopathology which needs to be established [30, 31]. The hamster LCMV-antibody response is most probably of secondary importance to the lethal or non-lethal outcome of acute infection. Cell-mediated immunity may regulate protective anti-viral mechanisms that are paramount to the health and survival of hamsters with LCMV-infection. Cell-mediated immune responses were described for hamsters with inapparent, non-fatal arenavirus infections [9, 28, 51]. Ongoing studies in our labo-

ratory have established a direct relationship between the resistance of hamster strains to lethal LCMV-infections and cell-mediated immune responsiveness to LCMV [30; Genovesi and Peters, unpublished results]. The results of these latter studies will be presented in a future communication.

The importance of T-lymphocyte mediated encephalitis, visceral-disease, and chronic immunecomplex disease in the murine-LCMV paradigms is unquestioned [7, 11, 23]. However, in several arenaviral disease models immunosuppressive treatment exacerbates clinical disease manifestations and death, rather than affording host protection: Pichinde virus in hamsters [6], selected Junin virus strains in rats, mice, and guinea pigs [4, 5, 12, 21], and LCMV in guinea pigs [36]. These models are more relevant to the arenaviral hemorrhagic fevers of man [30, 31] rather than the well studied murine-LCMV systems. Due to potential experimental flexibility, the hamster-LCMV models of acutely lethal-disease and non-lethal, inapparent, infections could serve as a useful system for further studies into the pathogenesis of human arenaviral hemorrhagic fevers.

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